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L6: Entry 1 of 38 File: USPT Jul 10, 2001

DOCUMENT-IDENTIFIER: US 6258599 B1
TITLE: Compositions and methods for treating viral infections

BSPR:
The amino acid sequences or peptides of interest fail to elicit an immune response in man through mimicry of epitopes on human and other proteins. Of particular interest are peptide epitopes shared between HIV proteins and human alpha fetoprotein, aspartyl protease, deoxyuridine 5'-triphosphate nucleotidohydrolase, eosinophil cationic protein, eosinophil-derived neurotoxin and ribonuclease 4 precursor and peptide epitope regions mimicked by neurotoxins from Bungaris Naja, Dendoaspis, Psudechis, or Androctonus Centruroides.

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L6: Entry 7 of 38 File: USPT Apr 4, 2000

DOCUMENT-IDENTIFIER: US 6045793 A
TITLE: Recombinant ribonuclease proteins

BSPR:
Non-cytotoxic human members of the RNase A superfamily linked to tumor associated antigens by chemical (Rybak et al.(1991) J. Biol. Chem 266, 1202-21207, Newton et al. (1992) J. Biol. Chem. 267, 19572-19578) or recombinant means (Rybak et al. Proc. Natl. Acad. Sci. U.S.A. 89, 3165, Newton et al. (1994) J Biol Chem. 269, 26739-26745 have been shown to offer a strategy for selectively killing tumor cells with less immunogenicity than current strategies employing plant and bacterial toxins Rybak, S. M. & Youle, R. J. (1991) Immunol. and Allergy Clinics of North America 11:2, 359-380. Human-derived ribonucleases of interest include eosinophil-derived neurotoxin (EDN) and angiogenin.

BSPR:
Humanized versions of our rOnc molecules are also described which graft portions of mammalian or human-derived RNases such as angiogenin or human eosinophil derived neurotoxin (EDN) to the rOnc-derived molecules. A preferred embodiment of the invention is a molecule where the amino terminal end of EDN is placed onto the amino terminal end of the rOnc molecules. The surprising properties of these hybrid proteins with regard to ribonuclease activity and in vitro anti-tumor effects are described.

DEPR:
Comparisons of the rOnc sequences provided here can be made to described sequences in the pancreatic RNase A superfamily. Many of such members are known and include, but are not limited to, frog lectin from Rana catesbeiana (Titani et al., Biochemistry 26:2189 (1987)); ONCONASE.RTM. (Ardelt, W. et al., J. Biol. Chem. 266:245 (1991)); eosinophil derived neurotoxin (EDN) (Rosenberg et al., supra); human eosinophil cationic protein (ECP) (Rosenberg et al., J. Exp. Med. 170:163 (1989)); angiogenin (Ang) (Fett, J. W. et al., Biochemistry 24:5480 (1985)); bovine seminal RNase (Preuss et al., Nuc. Acids. Res. 18:1057 (1990)); and bovine pancreatic RNase (Beintama et al., Prog. Biophys. Mol. Biol. 51:165 (1988)), references for all such proteins are incorporated by reference herein. Amino acid sequence alignment for such RNases are also set out in FIG. 4 and in Youle et al., Crit. Rev. Ther. Drug. Carrier Systems 10:1-28 (1993) and in U.S. patent application Ser. No. 08/125,462, which is incorporated by reference herein.

DEPR:

G. Structural Analysis of the hybrid RNases. Modeling the hybrid RNase was based on the alignment of the structures for Onc (Mosimann S. C., Ardelt W., James M. N. G., (1994), Refined 1.7 A X-ray crystallographic structure of P-30 protein, an amphibian ribonuclease with anti-tumor activity (J Mol Biol 236, 1141-1153) and EDN (Mosimann S. C., Newton D. L., Youle R. J., James M., X-ray crystallographic structure of recombinant eosinophil-derived neurotoxin at 1.83A resolution J Mol Biol). This and subsequent alignments were carried out using ALIGN (Satow Y., Cohen G. H., Padlan E. A., Davies D. R., (1986), J. Mol Biol 190, 593-604).

DEPR:
The structural basis for the marked differences in activity between the Gly and Asp containing hybrid RNases are not obvious from modeling these proteins especially since residue 26 is distant from the active site. When the highly homologous structure of RNase A complexed with a pentanucleotide (Fontecilla-Camps J. C., deLorens R., leDu M. H., Cuchillo C. M., (1994), J. Biol Chem 269, 21526-21531) was superimposed on the structure of the hybrid protein model, the nucleotide was observed also to be distant from the region of the mutation. However, the arrangement of the polynucleotide chain in the different RNases does not necessarily have to coincide. In the structure of EDN, a second sulfate ion was found in addition to the one in the active site (Mosimann S. C., Newton D. L., Youle R. J., James M., X-ray crystallographic structure of recombinant eosinophil-derived neurotoxin at 1.83A resolution J Mol Biol). This second sulfate is likely replacing a phosphate from the nucleotide to be cleaved, but no phosphate ion is located in the equivalent position in the RNase A-pentanucleotide complex. Moreover, one of the phosphates in this complex is forming a salt bridge with Lys-66, a residue which has no counterpart in Onc since it is located in a loop with a different topology in both molecules. Thus, whether the difference in enzymatic activity between the Asp and Gly mutants in the chimera is related to a change in the binding affinity for the substrate remains an open question.

DEPR:
Although the structural basis for the difference in the activities of the two EDN-Onc hybrids is not clear, the EDN-like behavior of the rEDN.sub.(1-21) rOncG26 hybrid can likely be attributed to the configuration of the N-terminal region since both the pyroglutamic acid in nOnc and Lys-1 in EDN are located in the area of the active site (Mosimann S. C., Ardelt W., James M. N. G., (1994), Refined 1.7 A X-ray crystallographic structure of P-30 protein, an amphibian ribonuclease with anti-tumor activity J Mol Biol 236, 1141-1153; Mosimann S. C., Newton D. L., Youle R. J., James M., X-ray crystallographic structure of recombinant eosinophil-derived neurotoxin at 1.83A resolution J Mol Biol). In addition, the introduction of a Gly mutation in [Met-(-1)]rOnc did not significantly affect enzymatic activity. The preference of U over C in the B1 subsite of RNase A has been related to the presence of a particular residue (Asp-83) (DelCardayre S. B., Raines R. T., (1995), A residue to residue hydrogen bond mediates the nucleotide specificity of ribonuclease

A J Mol Biol 252, 328-336). The corresponding residue in nOnc is also an aspartic acid (Asp-67), while in EDN this position is occupied by a histidine (His-82). EDN is more active toward poly (A,C), suggesting that it "prefers" C in the B1 subsite, possibly because it contains a histidine residue as opposed to the aspartic acid in nOnc and RNase A. Taken together, this could explain the decreased activity of the Gly containing hybrid relative to rEDN since, according to this hypothesis, the presence of the Asp residue contributed by the rOnc sequence would favor the binding of U over C. With regard to the difference in PRI inhibition, the superposition between the hybrid proteins and RNase A demonstrates that Asp-26 in the EDN-Onc chimeras is in the equivalent position to Asn-27 in RNase A that has been reported to be in contact with PRI (Kobe B., Deisenhofer J., (1995), Nature 374, 183-186). In addition, Asp-24 in both chimeras is very close to this region. Thus, the accumulation of negative charges in this area could prevent binding by the inhibitor. If so, the substitution of Gly for Asp would decrease the negative charge and restore the binding capacity.

CLPV:
wherein Met(-1) refers to an amino terminal residue of Met; wherein eosinophil derived neurotoxin.sub.(1-m) refers to a contiguous sequence of amino acids of a length beginning at amino acid position 1 of eosinophil derived neurotoxin (SEQ ID NO:9) and continuing to and including amino acid position "m" of eosinophil derived neurotoxin; wherein Onc.sub.(n-104) refers to a sequence of contiguous amino acids beginning at no acid position "n" and continuing to and including amino acid position 104 as set out in SEQ ID NO:1; and wherein "m" is the amino acid position of eosinophil derived neurotoxin selected from the group consisting of 5, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22; such that:

CLEQ:
Met(-1) eosinophil derived neurotoxin.sub.(1-m) Onc.sub.(n-104)

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USPT	(EDN same eosi$ adj derived adj neuro$) same pancreat$	0	L2
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